FREQUENCIES OF BLOOD GROUP ANTIGENS IN THE LUMBEE INDIANS OF NORTH CAROLINA

Wm Patrick Monaghan

Submitted to the Graduate School of Bowling Green State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

March 1974

Approved:

William O. Bopton Advisor

Frank R. Camp, Jr. Co-Advisor

Charles Shields

William D. Jann

ABSTRACT

The Lumbee Indians, who reside in and around Robeson County, North Carolina, have been described as a complex population that has lived in relative isolation for the past few hundred years. Their origin is unknown.

This blood group study was part of an ongoing population genetic study being completed by Dr. Everett K. Spees, Duke University, which will ultimately include the results from plasma protein markers and histocompatibility antigens. One hundred sixty-four blood samples were collected by venipuncture. The red cells were glycerolized and frozen until use. A detailed geneology was obtained for each individual studied in an effort to avoid biasing the gene frequencies by counting more than one member from each family.

The antigens studied in this paper included A, B, Rh_o, rh', rh", hr', hr", P, M, N, K, Fy^a, Di^a, Jk^a, Kp^a and Kp^b. All serums were tested for hepatitis-associated antigen (HbAg) and abnormal hemoglobin by electrophoresis. Gene frequencies were calculated and tested for goodness of fit to a Hardy-Weinberg equilibrium. A further statistical analysis was accomplished using a test for the equality of two proportions (gene frequencies) to compare the results of this study with others.

The results of this study were similar to those of the only other published study of these Indians. The gene frequencies of the blood groups studied indicate admixture from Black, White, and other Indian sources. All specimens tested were negative for hepatitis-associated antigen. The only abnormal hemoglobin variants found in 104 specimens were two unrelated samples which contained hemoglobin C. No sickle hemoglobin was found.

From the samples tested, it appears that the Lumbee Indians are a group of heterogenous composition. They show genetic similarity to the Tuscarora and White populations and dissimilarity to Cherokee, Negro and Seminole populations.

ACKNOWLEDGEMENTS

The author is most grateful for the advice, assistance and guidance given by Drs. William Baxter and William D. Hann in the compilation and editing of this manuscript. While accepting any shortcomings as my own, I wish to acknowledge the assistance of LCOL. Sherwood S. Hill, USAF, BSC, who not only stimulated my interest in this study, but further provided a great deal of effort in completing it.

The study would not have been possible without Dr. Everett K. Spees, COL. MC, USA, who performed the on-site study, collected the specimens, and led the complete project. Dr. D. B. Amos, Professor of Immunology of Duke University Medical Center, Durham, North Carolina was the sponsor of the field study. I also wish to thank those people in Robeson County who assisted in the actual gathering of information and samples. It included the local population representative, Mr. Adolf L. Dial, Head, Department of Indian Studies, Pembroke State University, Pembroke, North Carolina; Mr. Donald Freeman, the Laboratory assistant in the field, and the use of facilities in the Prospect Methodist Church for the laboratory space.

Most importantly, I wish to heartily thank COL. Frank R. Camp, Jr. MSC, USA of the U.S. Army Medical Research Laboratory, Fort Knox, Kentucky,

who not only provided the space and assistance for performing the serological tests, but who has further been my mentor and friend.

This study is dedicated to my lovely wife, Judy, who has overcome tremendous hardships and still found time to assist me in my educational pursuits.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

T

INTRODUCTION

The Lumbee Indians are a relatively isolated group of people numbering approximately 25,000 who live in and around Robeson County, North Carolina. They remain the only Indian group in North Carolina whose origin is unknown. It is believed by many historians that they are the descendants of the friendly Indians with whom the lost colonists of Sir Walter Raleigh's expedition found refuge (Rights 1957 and Stoutenburg 1960).

Evidence both for and against this attribution has been collected. In support of this theory is the fact that many of the names of these Indians are known to be the same as those of the lost colonists. Also, many unique pronunciations and idioms of the early English have been found in the Lumbee, although similar usages have been found in other isolated regions throughout the state. It is further known that many of the Lumbees adopted customs and manners of the new English settlers in that they built their own houses and cultivated their crops in ways similar to the English. The Lumbees are unusual in that they lack any remnant of their own ancient language.

The recorded history of these people is replete with many names for the group. The most widely used ones are "Indians of Robeson County"

or more recently "Lumbee Indians". Another name that had been closely associated with these people is "Croatan". This name comes from the initials CRO carved in a tree that was found when the English settlers returned to North Carolina to visit and replenish their colony.

An original Croatan tribe was known to have been located along the Outer banks of the Eastern seaboard. According to Rights (1957) they appear to have been associated with the much larger Hatteras tribe which populated that portion of the Atlantic coast. The name Croatan and its association to the Indians of Robeson County was originally applied to them by Hamilton McMillan. Mr. McMillan was an eminent lawyer in the county who befriended the Indians and helped plead their cause of official recognition before the General Assembly of North Carolina in 1885. He was the first person to record the possibility that these Indians could be the descendants of the lost colony. Whether or not this was common knowledge prior to this time is speculation. Apparently his belief that these Indians were related to the original Croatan tribe was based on his lifetime study of this group of Indians, their habits, known history, and folklore.

What motivated the remnants of the original tribe of Croatan Indians, located along the Atlantic coast, to leave the sandy banks and

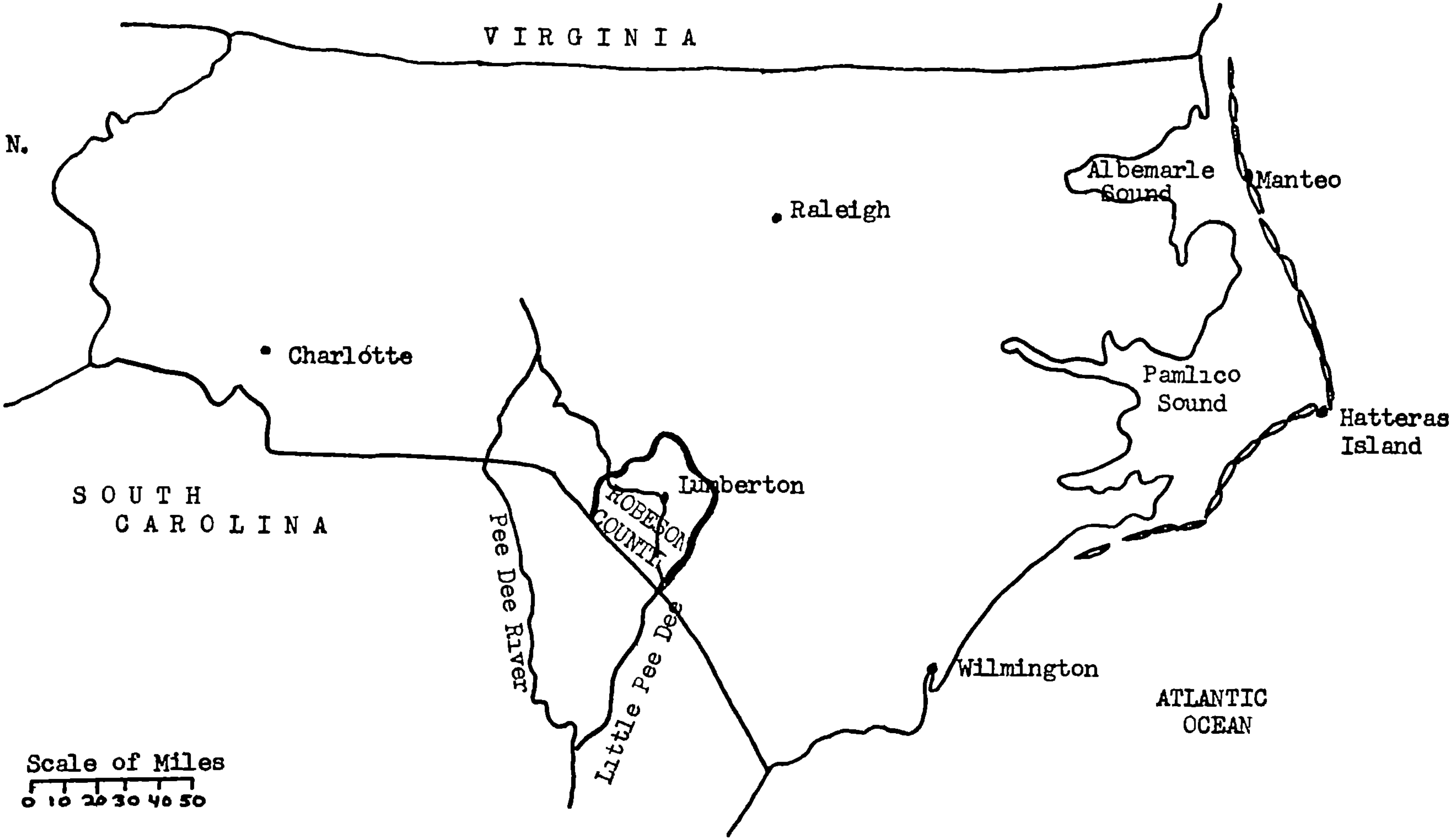
migrate to the southern interior is unknown. Probably encroachment and abuse by the early settlers caused them to leave their homes. They evidently traveled southward along the coast, and upon finding more settlers, turned inward along the rivers, streams, and swamps. They ultimately settled along the Lumber River (Figure 1).

Throughout recorded history the Lumbee Indians have remained relatively isolated. They thave lived along the Lumber River since the early 1700's; land grants date from 1732. Although they have never engaged in any open conflict with the white man, on a few occasions in the early 1700's they were known to have fired a few shots at surveyors who were mapping the territory.

In the early 1800's the Lumbees were mostly land owners, who attended public schools and were treated as whites. However, in 1834, along with some blacks and Cherokees, they were designated "Free Persons of Color" and were thereafter denied the right to vote, build churches or send their children to white schools (Rights 1957). For 50 years they went without schools rather than send their children to the inadequate negro schools. During this half century a strong anti-black bias developed, then in 1885 the General Assembly of North Carolina officially recognized them as Croatan Indians.

Figure 1

Map of North Carolina showing the relationship of the Outer banks to Robeson County



Their name was changed once again by the State Legislature of 1911 to the name of "Indians of Robeson County". Approximately two years later they were designated "Cherokee Indians of Robeson County". This label evoked considerable consternation and was often confused with the 5,000 member Eastern band of Cherokee Indians who live on a reservation at Cherokee, North Carolina. Finally, the 84th Congress of the State of North Carolina on May 22, 1956 officially changed their name to the Lumbee Indians, which because of this official recognition seems to be the most appropriate current designation for the group.

It is rare to find a group of people who have lived as a complex population isolate of undetermined origin for such a long period of time. It is a basic immunological principle that an individual's genetic endowment may be partially determined by the various blood group antigens as detected by appropriate antisera (American Association of Blood Banks 1970). In order to examine this unique racial and geographical isolate in terms of those antigen systems that lend themselves to distinctive differences between various groups of people, samples of Lumbee Indian blood were collected. This paper reports the results of tests for the presence of various red cell antigens. Also included are the results of tests for hepatitis-associated antigen and abnormal hemoglobins.

The present investigation was part of a larger study being conducted by Dr. E. K. Spees in conjunction with Dr. D. B. Amos, Professor of Immunology, Duke University Medical Center, Durham, North Carolina. The total study will also include such genetic markers as histocompatibility antigens and plasma protein antigens.

The only previous study of Lumbee blood group frequencies was conducted by Pollitzer in 1964. His study of the ABO, Rh and Kell Systems was limited to school children in Lumberton, North Carolina. Although his study was larger than the present one, there appeared to be no effort to trace the ancestry and kinship of the individuals sampled.

The present study tended to concentrate on the older Lumbee Indians who were more geographically isolated and agriculturally oriented. Among the metropolitan Lumbees, the people in our study are often referred to as the "Swamp Lumbees". Detailed geneologies were collected to avoid skewing the antigen frequency data by the inclusion of more than one member from each family. Other information gathered in this study yielded blood group antigen frequencies for antigen systems not studied by Pollitzer. Analysis of our more comprehensive blodd group data should aid in understanding the complex origin and ancestry of this group of Indians.

MATERIALS AND METHODS

Blood samples were collected by venipuncture during August, 1972 from 164 individuals. Samples were obtained in and around Pembroke, North Carolina. An oral interview was conducted and a detailed geneology was collected on the same day (Appendix F). This interview was attended by a prominent member of the Lumbee community who often helped identify other relationships in the study. On many occasions headstones at the local cemetry were checked to verify the data. The information collected included name, sex, age, date, and place of birth, mother and father's name, place and date of birth, death, number of children and kinships. A brief review of each individual's history was also made to see if any prominent diseases were known to afflict more than one family member. Pedigree charts were constructed from this data. Geneologies were carried to three generations. Consanguinity, defined as the offspring resulting from the marriage of first cousins or more immediate family, was denied by all subjects interviewed. Any person beyond first cousin was considered not related for analysis of blood group frequency data. Approximately 130 individuals fit this criterion.

The age of individuals in our sample ranged from 14 to 99 years. Approximately 70% of the sample group were over 50 years and many were

septuagenarians and octogenarians. Only a few individuals under 30 years were included in the study. The male to female ratio was approximately 60/40.

The venous blood samples were collected in standard 7cc Becton Dickinson, non-anticoagulant, vacutainer tubes. They were defibrinated with the use of 6-10 glass beads and the blood was passed through a nylon filter to remove the granulocytes. The blood was layered on a Ficoll-Hypaque gradient, centrifuged at 500 x g for approximately 5 min. to create a serum-cell interface, and the serum was removed and frozen (Perper et al. 1968). The buffy coat, composed of lymphocytes, was removed and brought to a final concentration of 10% dimethylsulfoxide (DMSO) in Minimal Essential Medium (MEM). The lymphocyte suspensions were frozen in liquid nitrogen for later histocompatibility studies (Stoppford et al. 1972).

The erythrocytes were washed twice with 0.85% NaCl, packed and mixed one to one with glycerol preservative solution (40% glycerol in potassium citrate buffer). The cells were frozen, shipped on dry ice and kept in a Revco freezer at -70°C until use.

Prior to antigen testing the red cells were thawed, deglycerolized by dialysis with three different NaCl concentrations (Huggins et al. 1971),

washed three times with 0.85% NaCl, resuspended and stored in a glucose, citrate, phosphate preservative medium (Burgess and Voss 1971) at 4°C. All of the red cell antigen tests utilized commercial typing serums and were performed using the directions accompanying each serum or standard procedures as specified by the American Association of Blood Banks (1970). The blood group antigens investigated were A, B, M, N, P, rh', hr', Rh_o, rh", hr", Kell, Kp^a, Kp^b, Fy^a, Di^a, Di^b and Jk^a. This paper will utilize the nomenclature suggested by the American Association of Blood Banks (1970).

The gene frequencies for the ABO system were calculated by the Bernstein method with correction factor (Bernstein 1930). The Rh gene frequencies were determined by Wiener's methodology (Wiener and Wexler 1958). All other gene frequencies were determined by direct count. Chi-square tests were performed to measure the goodness of fit between the observed values and those expected in a Hardy-Weinberg equilibrium (Camp et al. 1971). Gene frequencies of Lumbee Indians were compared with those of other racial groups by a test for the significance of differences between two proportions (Sokal and Rohlf 1969).

Hepatitis-associated antigen (HBAg) testing of the serums was performed by counter electrophoresis (Spectra Biologicals 1971). Red

cell hemolysates were prepared and tested for the presence of abnormal hemoglobin by cellulose acetate electrophoresis (Helena Laboratories). The antiserum used for the test did not list the specificity other than being anti-HBAg. It was probably what is now known as a polyvalent mixture of anti-HB_s and anti-HB_c (Center for Disease Control 1974).

RESULTS

The results are subdivided by systems. The variation in the number of samples from system to system was due mainly to the loss of cells occurring either upon freezing, storage, deglycerolization, or during the actual testing procedure. Those samples that failed to yield clear-cut results and could not be rechecked, owing either to loss of cells or too few cells, were not tabulated in the phenotype frequencies.

ABO system

The results of field testing of A, B, and Rh_o antigens by a slide method were compared to those obtained in the laboratory using the tube tests. Of 126 slide typings there were three discrepancies in the ABO system and one with the Rh_o antigen when the frozen-thawed cells were tube tested. These discrepancies were not counted in the gene frequency calculations.

All specimens that were phenotypically A or AB were typed for subgroups using Anti-A_1 lectin prepared from the seeds of Dolichos biflorus. Only one sample, an AB, was found not to react with Anti-A_1 lectin and was listed as A_2B. On examination of pedigree, however, this person was found to be related to an individual previously counted in our sample. Therefore, this sample was not included in the phenotype frequencies of unrelated donors. No other subgroups of A or B antigens were noted.

Table I lists the A, B, O and AB phenotypes for the unrelated donors, their computed genotypes, and results of analysis of the data by statistical tests. Table II shows the same data when all ABO groups were counted without regard to donor relationship. The apparent higher O gene and slightly lower A gene frequencies in the larger samples were not statistically significant when tested for the equality of two proportions (Sokal and Rohlf 1969). Both sets of data fit a Hardy-Weinberg equilibrium.

Rh system

The distribution of Rh genotypes and phenotypes is depicted in Table III. The Wiener theory of inheritance was utilized, primarily because of the lack of an Rh_o reciprocal, and the ability of this theory to explain such phenomena as Rh mosaicism and compound Rh genes.

The specimens were not tested for the presence of rh^{w1} antigen. However, the $\mathfrak{R}h_o$(Du) frequency was 1.6% (2 in 126). Both of these samples appeared to be of the gene interaction or "high grade D^u" type and the most probable genotype was $\mathfrak{R}_1r'$. In testing for the presence of $\mathfrak{R}h_o$ 12 additional specimens were noted to give weakly positive reactions with anti-Rh_o antisera. These specimens were subjected to the $\mathfrak{R}h_o$ test for the purpose of enhancement of reaction. Reactions in

TABLE I

ABO phenotypes and gene frequencies of unrelated Lumbee Indians

Phenotype	Number	Frequency
O	64	.5079
A	47	.3730
B	13	.1032
AB	2	.0159
TOTAL	126	1.000

Genes	Frequency[a]
A	.2187
B	.0622
O	.7190

$\chi^2_{(1)} = 0.786$[b]

$.25 < P < .5$

[a]adjusted by Bernstein method with correction (Cavalli-Sforza and Bodmer 1971)

[b]test for goodness of fit to a Hardy-Weinberg equilibrium.

TABLE II

ABO phenotypes and gene frequencies of related and unrelated Lumbee Indians

Phenotype	Number	Frequency
O	90	.5488
A	52	.3171
B	19	.1158
AB	2	.0122
A_2B	1	.0061
TOTAL	164	1.000

Genes	Frequency[a]
$A + A_2$	.1855
B	.0692
O	.7453

$X^2_{(1)} = 0.475$[b]

$.5 < P < .9$

[a]adjusted by Bernstein method with correction (Cavalli-Sforza and Bodmer 1971)

[b]test for goodness of fit to a Hardy-Weinberg equilibrium.

TABLE III

Rh phenotypes and gene frequencies in the Lumbee Indians

Phenotypes	Number	Proportions
Rh_1Rh_1	10	.1205
Rh_1rh'	2	.0242
Rh_zRh_2	1	.0120
Rh_1Rh_2	8	.0964
Rh_1rh	33	.3976
Rh_2Rh_2	5	.0602
Rh_2rh	8	.0964
Rh_orh	14	.1687
rh rh	1	.0120
rh'rh	1	.0120
TOTAL	83	1.000

Genes	Frequency[a]
r	.1042
r'	.0411
r"	.0000
R^o	.3111
R^1	.3822
R^2	.1613

[a](Wiener and Wexler 1958) formulas employed are listed in Appendix D.

all cases went from a weak ± to greater than 2+. The most probable genotypes of these 12 individuals are listed in Appendix B.

When all specimens were tabulated without regard for kinship, the frequency of Rh_o positive was .9603 and .0397 for Rh_o negative. This compares closely with the results in Table III which show a phenotype frequency of .9760 for Rh_o positive and .0240 for Rh_o negative for unrelated donors.

<u>MN system</u>

The results of M and N antigen testing and calculated gene frequencies are given in Table IV.

The S and s antigens were not tested, and Anti-M_1^a was not available to test for homozygous N types for this rare antigen linked to the MNSs locus.

The gene frequencies were derived by gene counting and the Hardy-Weinberg equilibrium computation was performed according to those procedures given in Camp (1971) and Race and Sanger (1968). The analysis of eight additional samples that were excluded based on kinship, changed the gene frequency slightly; the N gene increased from .3878 to .4035.

The chi-square value for testing goodness of fit to a Hardy-Weinberg equilibrium was $X^2(1) = 4.11$ This significant difference was caused by a greater than expected number of heterozygotes.

TABLE IV

MN phenotypes and gene frequencies in the Lumbee Indians

Phenotypes	Number	Frequency
M	15	.3061
MN	30	.6123
N	4	.0816
TOTAL	49	1.000

Genes	Frequency
M	.6122
N	.3878

$\chi^2_{(1)} = 4.11$[a]

$P < .05$

[a]test for goodness of fit to a Hardy-Weinberg equilibrium.

P system

The P antigens were analysed with two antiserums P and P_1, which defined the two phenotypes P_1 and P_2. None of the other P phenotypes p, $p^k{}_1$, and $p^k{}_2$ were noted in our sample. The usual variability in reactivity of the P_1 positive red cells was noted and doubtful reactions were not counted in the calculations of phenotypes. The results of the testing are listed in Table V.

Duffy system

Only Anti-Fy^a was available for typing in the Duffy system. The results are given in Table VI, and show 53% of the sample to be positive for the Fy^a antigen. This system showed a significant difference ($P<.05$) when both related and unrelated samples were computed in the phenotype frequency. The percentage of phenotype Fy^a negative increased from 47% to 66%.

The gene frequencies were computed by taking the square of the differences of direct counts.

Kell system

All bloods were typed with K, Kp^a and Kp^b antiserums. The results of this testing are shown in Table VII.

Kidd system

The only available antiserum, Anti-Jk^a, reacted with 86% of the individuals tested and gave a gene frequency of .6265 (Table VIII).

TABLE V

P phenotypes and gene frequencies in the Lumbee Indians

Phenotype	Number	Frequency
$P + P_1$	32	.8205
P_2	7	.1795
TOTAL	39	1.000

Genes	Frequency
P_1	.5897
P_2	.4103

TABLE VI

Duffy phenotypes and gene frequencies
in the Lumbee Indians

Phenotype	Number	Frequency
Fy^a+	24	.5333
Fy^a-	21	.4667
TOTAL	45	1.000

Genes	Frequency
Fy^a	.3169
Fy^b	.6831

TABLE VII
Kell phenotypes and gene frequencies
in the Lumbee Indians

Phenotype	Number	Frequency
$K - Kp^a - Kp^b +$	42	.9130
$K + Kp^a - Kp^b +$	4	.0870
$K - Kp^a + Kp^b -$	0	.0000
TOTAL	46	1.000

Genes	Frequency
$K\ Kp^a$	.0000
$K\ Kp^b$	.0445
$k\ Kp^a$	.0000
$k\ Kp^b$	.9555

TABLE VIII

Kidd Phenotypes and gene frequencies in the Lumbee Indians

Phenotype	Number	Frequency
$Jk^{a}+$	37	.8605
$Jk^{a}-$	6	.1395
TOTAL	43	1.000

Genes	Frequency
Jk^{a}	.6265
Jk^{b}	.3735

Diego system

Of the 48 unrelated people tested, 98% (47) were negative with Anti-Di^a as shown in Table IX. Two other individuals were found to have the Di^a antigen but were related to other members in the study. When the combined samples of related and unrelated individuals were counted, a 5.3% phenotype frequency of Di^a resulted.

Other testings

All 91 specimens of serum tested for the presence of hepatitis associated antigen (HBAg) were negative by counter electrophoresis.

One hundred four of the original specimens were examined for the presence of abnormal hemoglobin by cellulose acetate electrophoresis. The results of the analysis are shown in Table X. No sickle hemoglobin was found. Two specimens contained hemoglobin C. The two strips were cleared and found to contain 22% and 16% hemoglobin C by densitometric analysis. The only other components noted in the densitometer scans of these strips were hemoglobin A and a small amount of hemoglobin F (<1%).

TABLE IX

Diego phenotypes and gene frequencies in the Lumbee Indians

Phenotype	Number	Frequency
Di (a-b-)	0	.0000
Di (a-b+)	47	.9792
Di (a+b+)	1	.0208
Di (a+b-)	0	.0000
TOTAL	48	1.000

Genes	Frequency
Di^a	.0105
Di^b	.9895

TABLE X

Results of Hemoglobin Electrophoresis

Phenotype		Number	Frequency
AA		102	.9807
AC		2	.0193
AS		0	.0000
SS		0	.0000
	TOTAL	104	1.0000

DISCUSSION

The results of the blood type analysis of the Lumbee Indians may be more fully appreciated by comparing them with frequencies reported for other racial groups that may have contributed parental genes. Table XI enables one to see the diversity of genotype frequencies among several Indian groups and representative White and Negro populations. Examination of the table emphasizes the anthropological value of studying blood group diversity in human populations by analysing the differences in a relatively large number of genes; in several cases as many as 22. The table includes results of statistical tests of the differences in gene frequencies between the Lumbee's and the other groups listed (Sokal and Rohlf 1969).

Comparison of the results in the ABO system with those of Pollitzer's previous study of the Lumbees (Hybrid) shows close agreement. His published phenotype frequencies of 50% - O; 35% - A; 11% - B; 3% - AB are strikingly similar to ours (Table I).

The Lumbee Rh gene frequencies were similar to those in the other groups listed in Table XI, with the genes R^z, r'', r' and r^y being rare or completely absent. The frequency of r gene was relatively high but only half the frequency previously reported by Pollitzer (1964). The frequent occurrence of the R^o gene (31%) in the Lumbee when compared

TABLE XI

Allele frequencies in Lumbee Indians and other populations which may have contributed parental genes

Allele	Lumbee[a] N=39-126	Cherokee[b] (full blood) N=78		Cherokee[b] (mixed) N=94		Tuscarora[c] (combined) N=53		Hybrid[d] (Lumbee) N=54-1273		Seminole[e] N=224		Negro[f] N=119-515		White[g] N=1000-8767	
O	0.713	0.981	***[h]	0.884	**	0.713	n.s.	0.709	n.s.	0.923	***	0.710	n.s.	0.660	n.s.
A_1	0.218	0.000	***	0.071	**	0.235	n.s.	0.218	n.s.	0.036	***	0.137	*	0.209	n.s.
A_2	0.000	0.006	n.s.	0.023	*	0.013	n.s.	0	n.s.	0.002	n.s.	0	n.s.	0.069	***
B	0.062	0.013	n.s.	0.022	n.s.	0.039	n.s.	0.073	n.s.	0.039	n.s.	0.153	**	0.061	n.s.
R^z(CDE)	0	0.058	**	0.021	n.s.	0.019	n.s.	0	n.s.	0.072	***	0.004	n.s.	0.002	n.s.
r (cde)	0.104	0[i]	***	0[i]	***	0.123	n.s.	0.227	**	0.135	n.s.	0.257	***	0.389	***
R^1(CDe)	0.382	0.615	**	0.670	***	0.566	*	0.294	n.s.	0.433	n.s.	0.087	***	0.408	n.s.
r''(cdE)	0	0	n.s.	0	n.s.	0	n.s.	0	n.s.	0	n.s.	0.004	n.s.	0.012	*
r'(Cde)	0.041	0	**	0	**	0	*	0	**	0.015	n.s.	0.022	n.s.	0.011	n.s.
R^2(cDE)	0.161	0.228	n.s.	0.245	n.s.	0.292	n.s.	0.081	n.s.	0.435	***	0.069	*	0.141	n.s.
r^y(CdE)	0	0	n.s.	0	n.s.	0	n.s.	0	n.s.	0	n.s.	0	n.s.	0	n.s.
R^o(cDe)	0.311	0.039[i]	***	0.064[i]	***	0	***	0.370	n.s.	0	***	0.558	***	0.026	***

[a] present study
[b] Pollitzer et al. 1962
[c] Mohn et al. 1963
[d] Pollitzer 1964
[e] Pollitzer et al. 1970
[f] Pollitzer 1958
[g] Race and Sanger 1968
[h] Test for equality of proportions (Lumbee vs. other groups) at infinite degrees of freedom (Sokal and Rohlf 1969)
[i] R^o and r alleles were counted together because of the inability to differentiate these genes in the absence of the corresponding phenotypes

* .0<P<.05
** .001<P<.01
*** <P .001
n.s. not significant(P>.05)
n.t. not tested

TABLE XI, Continued

Allele	Lumbee N=39-126	Cherokee (full blood) N=78		Cherokee (mixed) N=94		Tuscarora (combined) N=53		Hybrid (Lumbee) N=54-1273		Seminole N=224		Negro N=119-515		White N=1000-8767	
M	0.612	0.801	*	0.686	n.s.	0.642	n.s.	0.546	n.s.	0.786	*	0.509	n.s.	0.532	n.s.
N	0.388	0.199	*	0.314	n.s.	0.358	n.s.	0.454	n.s.	0.214	*	0.491	n.s.	0.468	n.s.
P_1	0.590	0.592	n.s.	0.601	n.s.	n.t.		n.t.		0.491	n.s.	n.t.		0.460	n.s.
P_2	0.410	0.408	n.s.	0.399	n.s.	n.t.		n.t.		0.509	n.s.	n.t.		0.540	n.s.
Jk^a	0.627	0.469	n.s.	0.457	n.s.	n.t.		n.t.		n.t.		n.t.		0.514	n.s.
Fy^a	0.317	0.547	*	0.658	***	n.t.		0.293	n.s.	0.666	***	0.016	***	0.421	n.s.
K	0.045	0.006	n.s.	0.005	n.s.	0	*	0.028	n.s.	0	**	0.013	n.s.	0.046	n.s.
Kp^a	0	0	n.s.	0.031	*	n.t.		n.t.		0.000	n.s.	n.t.		0.023	*
Kp^b	1.000	1.000	n.s.	0.963	*	n.t.		n.t.		1.000	n.s.	n.t.		0.977	*
Di^a	0.011	0	n.s.	0	n.s.	0.058	n.s.	n.t.		n.t.		n.t.		0.010	n.s.
Di^b	0.989	n.t.		n.t.		0.942	n.s.	n.t.		n.t.		n.t.		0.990	n.s.

to the non-Lumbee Indian populations was perplexing. The R^o gene has a reported frequency of 38% to 96% in Negro populations (Terasaki 1970). The elevated frequency of this gene in the Lumbee Indian may reflect previous black admixture. However, the R^o gene has been reported to havea high frequency in Indian groups other than those used for comparison in this study, particularly in various Mexican Indians (Cordova et al. 1967).

The M and N gene frequencies in the Lumbee compared quite well with the frequencies of these genes in other Indian groups (mixed Cherokee and Tuscarora) which have undergone admixture with non-Indian populations. Our sample did not show a good fit when tested against Hardy-Weinberg equilibrium, owing to a greater than expected number of MN heterozygotes. Since this was the only instance of departure from an equilibrium, it is likely a chance occurrence and not suggestive of significant flux in the genetic pool of the Lumbees.

The Lumbee P system genes were similar in frequency to those reported in the Cherokee and Seminole. A high percentage of P_1 was not found, which argues against extensive admixture with Negroes (Adams and Ward 1973).

The frequency of Duffy antigen, Fy^a, shows a wide range of frequency in the various Indian groups used for comparison. It is infrequent or absent in Negro populations (Adams and Ward 1973). The frequency of this gene in our study compares very closely with the frequency previously reported in Lumbees by Pollitzer (1964).

A frequency of 0.627 for the Jk^a antigen is comparatively high and might suggest negro admixture.

The Kell system, inherited as three pairs of allelic characters, (K and k; Kp^a and Kp^b; Js^a and Js^b) allows for reasonable distinction between different populations. Unfortunately, one of the more discriminating antiserums, Anti-Js^a, was not available for testing. The K antigen has been described as occurring in 9% of the American Whites; 2% of the American Negroes; 1.2% in the Cherokees of Western North Carolina and 0% in the Tuscarora Indians (Zmijewski and Fletcher 1972). The incidence of 8.7% in the Lumbee Indians could indicate a substantial amount of white admixture. The Penny (Kp^a) and Rautenberg (Kp^b) antigen frequencies were also consistent with those reported in other Indian population groups.

The Diego blood group antigen, Di^a, shows a wide range of frequencies, from 36% in the Carib Indians to its absence in Eskimos and Cherokee

Indians (Race and Sanger 1968). Di^a frequency has been reported at 11% in the Chippewa Indians (Lewis et al. 1956) Mohn (1963) reports a frequency of 11.3% in the Tuscarora Indians, who at one time were known to have inhabited the area of the Carolinas. In our sample of unrelated Lumbees, the Di^a antigen frequency was 2%. Such an intermediate level could indicate admixture from those Indian sources relatively high in Di^a or dilution by genes from white or other populations of low frequency.

The hemoglobin electrophoresis analysis failed to show the presence of any hemoglobin S. Two hemoglobin C traits were recorded in 104 samples. This finding is in contrast to a previous report of 1.7% hemoglobin S and an equal amount of hemoglobin C in a sample of 1332 Lumbees (Pollitzer 1964). The lower frequency of hemoglobin S in our sample might be accounted for by the difference in sample sizes. Although another possibility could be the lack of skewing of gene frequencies in our sample.

Comparison of phenotype frequencies of unrelated donors with those from related donors was an effort to show how failure to take kinship into account might skew the observed frequencies. The ABO phenotypes listed in Table I when compared with those in Table II showed no

significant difference. Although kinship had no affect on the ABO frequencies it is possible to show that it was influential in other blood group systems. The Diego (Di^a) gene frequency increased from 2.0% to 5.3% when relationship was not considered. In another two allele system, Duffy, kinship resulted in a greater difference with the Fy^a negative phenotype increasing from 47% to 66% in related donors.

Many of the pedigree charts obtained in our sample emphasize the reason for considering kinship in the tabulation of gene frequencies. Figures 2 and 3, representative pedigree charts obtained during this study, illustrate the large families characteristic of the Lumbees sampled. If more than one member from each family were commonly included in the calculation of gene frequency the results would be considered biased.

The pedigree charts also indicate the lack of consanguinity and the ability of individuals to trace their family history a great distance. The incidence of illegitimacy was undetermined, but family studies suggest a lack of it.

The pedigree illustrated in Figure 3 shows the inheritance of a nervous affliction that manifested itself by uncontrollable tremors of

Figure 2

Family pedigree of a Lumbee Indian which indicates a typical expansive fertility

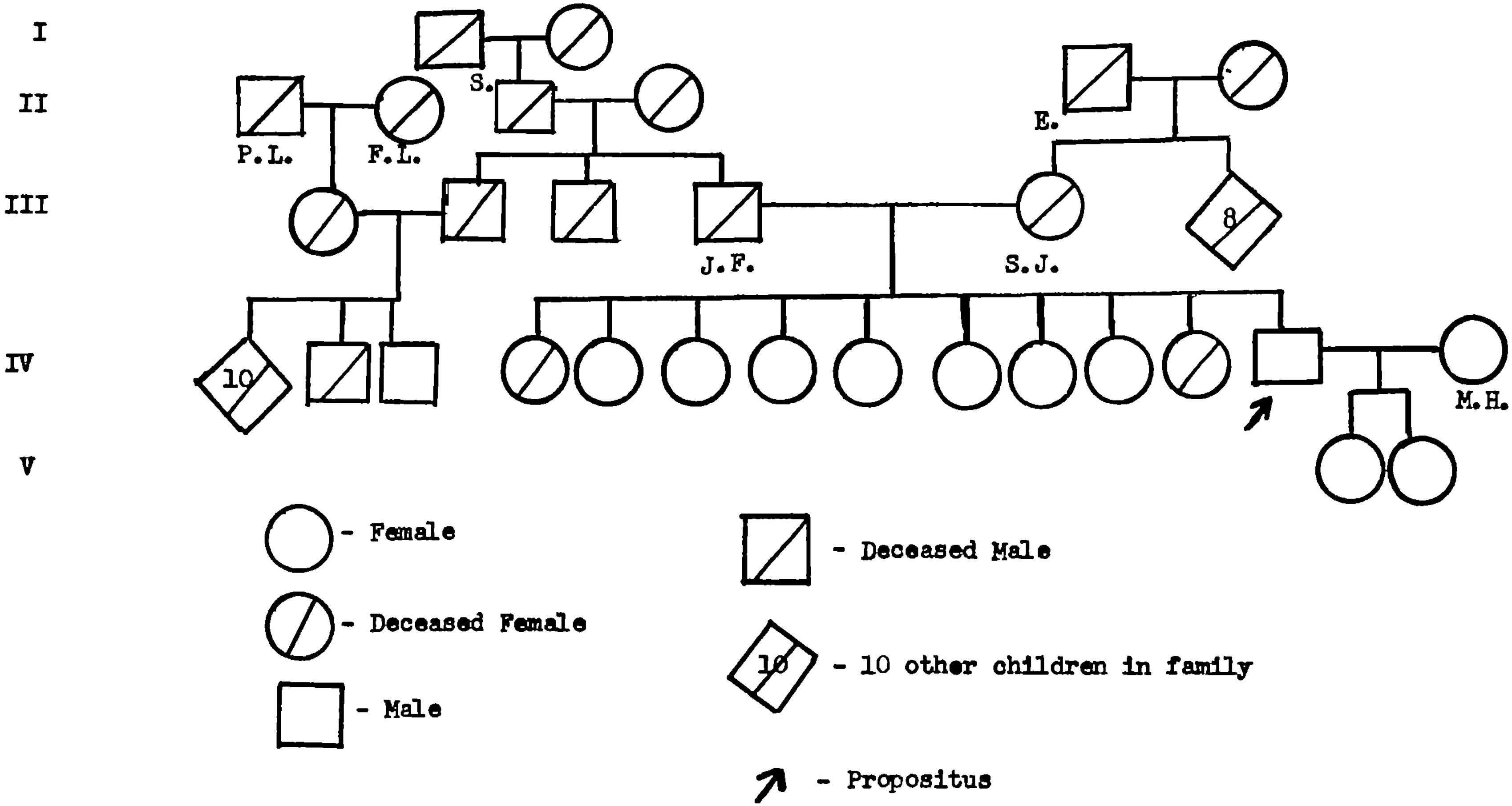
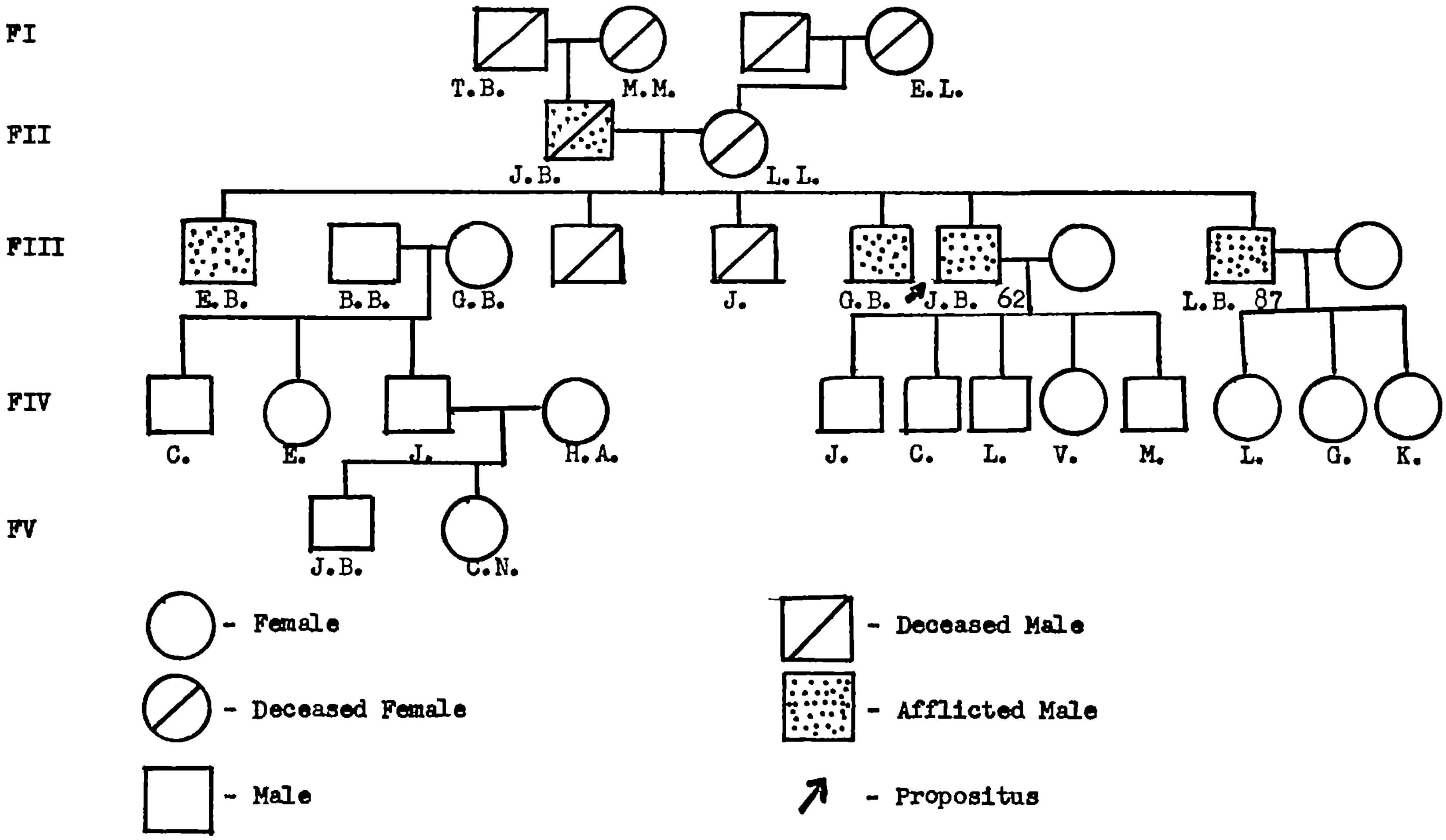


Figure 3

Pedigree of a Lumbee Indian Family showing the inheritance pattern of a nervous affliction



the hands. This affliction is apparently sex linked and did not become evident until after the age of 50. Another medical condition, noticeable on interview, was a relatively common occurrence of diabetes (Spees, personal communication).

Questions concerning the origin of the Lumbee Indians remain to be considered. Based on the results of statistical tests in Table XI, an index of dissimilarity was computed as the proportion of statistically significant differences ($P < .05$) in allele frequency between the Lumbee and other racial groups divided by the total number of pairwise tests possible (Table XII). The use of significant differences was deemed a more appropriate criterion since similarities between frequencies could be accounted for by such variables as sample size.

Inspection of the dissimilarity indices (Table XII) shows the Hybrid (Lumbee) group studied by Pollitzer in 1964 to be the least different from our sample. The small dissimilarity index of 0.12 would be expected since the only differences between our study and his were his sample size and the inclusion of many metropolitan Lumbees.

The analysis of blood group frequencies fails to support the idea that the Lumbees are an offshoot of the Cherokee Indians of North Carolina. Negro and Seminole groups also appear not to have made significant genetic contributions to the Lumbee Indians.

TABLE XII

Dissimilarity of allele frequencies between Lumbee Indians and other racial groups

Population [a]	Dissimilarity[b] index
Hybrid (Lumbee)	.12 (2/16)
Tuscarora	.24 (4/17)
White	.27 (6/22)
Negro	.43 (7/16)
Cherokee (mixed)	.45 (10/22)
Cherokee	.45 (10/22)
Seminole	.45 (9/20)

[a] same groups as those of Table XI

[b] proportion of statistically significant differences ($P < .05$) in allele frequency between the Lumbee and other racial group divided by the total number of pairwise tests possible.

The relatively low level of dissimilarity of blood group frequencies in White and Tuscarora populations as compared to the Lumbee supports the hypothesis that the Lumbee could have undergone admixture with early white settlers and Tuscarora Indians, known to have been indigenous to that portion of the country.

The completion of the histocompatibility antigen testing and plasma protein typing should augment the red cell antigen information presented in this paper and provide more definitive evidence concerning the origin of the Lumbees.

REFERENCES

Adams, J. and R.H. Ward. 1973. Admixture studies and the detection of selection. Science 180: 1137-1142.

American Association of Blood Banks. 1970. Technical methods and procedures, 5th ed. Chicago, Ill. 301 pp.

Bernstein, F. 1930. Fortgesetzte Untersuchungen aus der Theorie der Blutgruppen.Z. indukt abstramm.u. VererbLehre 56:233.

Burgess, B.J. and G. H. Voss. 1971. The preservation of laboratory panel cells. Vox sang 21:109-114.

Camp, F.R., Jr., N.F. Conte and F.R. Ellis. 1971. Genetics for the reference and forensic testing laboratory, a monograph. U.S. Army Medical Research Laboratory, Fort Knox, Ky. 98 pp.

Cavalli-Sforza, L. L. and W. F. Bodmer. 1971. The genetics of human populations. W. H. Freeman Co., San Francisco. 965 pp.

Center for Disease Control. 1974. Morbidity and mortality. 23:30.

Cordova, M.S., R. Lisker and A. Loria. 1967. Studies on several genetic hematological traits of the Mexican population. Am.J.Phys.Anthrop. 26:55-65.

Evans, W. McKee. 1971. To die game. Louisiana State University Press, Batan Rouge, La. 267 pp.

Helena Laboratories. Titan III. Beaumont, Texas.

Huggins, C. E., E. G. Cravalho, K.R. Diller, W. L. Watson and M. J. Seiler. 1971. Three stage dialysis for removal of glycerol from small aliquots of thawed frozen blood. Proc. Ann. Meet. Am. Assoc. Blood Banks. (Abstr) 24:17.

Lewis, M., H. Ayukawa, B. Chown, and P. Levine. 1956. The blood group antigen diego in North American Indians and in Japanese. Nature (London) 177:1084.

Mohn, J.F., R.M. Lambert and C. M. Zmijewski. 1963. Incidence of the blood group antigen Di^a in the Tuscarora Indians of North America. Nature. (London) 198:697.

Perper, R.J., T. W. Zee and M. M. Mickelson. 1968. Purification of lymphocytes and platelets by gradient centrifugation. J.Lab.Clin.Med. 72:842-848.

Pollitzer, W. S. 1958. The Negroes of Charleston, S. C. A study of hemoglobin types, serology and morphology. Am.J.Phys.Anthrop. 16:241-263.

Pollitzer, W.S. 1964. Analysis of a tri-racial isolate. Human Biol. 36:226-272.

Pollitzer, W.S., R.C. Hartman, H. Moore, R. E. Rosenfield, H. Smith, S. Hakin, P.J. Schmidt and W. C. Leyson. 1962. Blood types of the Cherokee Indians. AmJ.Phys.Anthrop. 20:33-43.

Pollitzer, W.S., K.K. Namboodiri, R. C. Elston, W.H. Brown and W.C. Leyshon. 1970. The Seminole Indians of Oklahoma, morphology and serology. Am.J.Phys.Anthrop. 33:15-29.

Race, R. R. and R. Sanger. 1968. Blood groups in man, 5th ed. F.A. Davis Co. Philadelphia. 599 pp.

Rights, D. L. 1957. The American Indian in North Carolina, 2nd ed. John F. Blair, Pub. Winston Salem. 320 pp.

Robeson County Church and Community Center. 1972. Facts about our Robeson County, Lumberton, North Carolina. 6 pp.

Sokal, R. R. and F.J. Rohlf. 1969. Biometry. W. H. Freeman and Co. San Francisco. 776 pp.

Spectra Biologicals. 1971. HAA testing by counter electrophoresis. Spectra Biologicals, Oxnard, Cal.

Stoppford, C.R., J.M.MacQueen, D.B. Amos and F.E. Ward. 1972. Some variations in lymphocyte freezing methods which do not affect cell viability. 2:22.

Stoutenburgh, J.L., Jr. 1960. Lumbee Indians. pages 220-221 in Dictionary of the American Indian. New York Philosophical Library, New York.

Terasaki, P. 1970. Other polymorphism chain frequencies. Histocompatibility testing 1970. Munksgaard, Copenhagen. 295 pp.

Wiener, A.S. and I.B. Wexler. 1958. Heredity of the blood groups. Grune and Stratton, New York. 150 pp.

Zmijewski, C. and J. Fletcher. 1972. Immunology, 2nd ed. Appleton-Century Crofts, New York. 168 pp.

APPENDIX A

Present status of Lumbee Indians in Robeson County with a description of the "Lowrie Tradition"

The present status of the Lumbee Indians, who live in the semicoastal lowland of Robeson County, is one of being economically depressed and that of living in an area of North Carolina that is still under developed. The county, one of the largest in the state, is composed of a tri-racial population of 91,000 inhabitants, of which 42% are White, 31% are Indian and 27% are Black. An indication of the county's dismal situation can be reflected in the statistics collected for the Office of Economic Opportunity in 1970 which revealed that 39% of the families fall within the poverty bracket; 25% of the people are functionally illiterate; 65% of the public school students drop out prior to graduation. The county has some service agencies, such as an O.E.O. program, mental health clinic, and a county church and community center. However, there is no Red Cross chapter, no Community Fund or Community Chest or Salvation Army type organization represented (Robeson County Church and Community Center 1972).

The Lumbee Indians, in representing over a third of the county, are the recepients of an even greater percentage of those statistics indicated.

This is indeed unfortunate in that the history and tradition of these people indicate that they have consistently provided many of their able men to the service. Historically, their men participated with Colonel Barnwell in his campaign against the Tuscarora Indians. Furthermore, some of them were recorded as serving in the Continental Army during the Revolution. They were further represented in the war of 1812, Civil War, both World Wars, as well as the more recent ones (Rights 1957).

It was during the Civil War that an unprecedented tradition arose. Many of their group were forced by the Confederacy to work along with the slaves on such embattlements as those in Wilmington, North Carolina and elsewhere away from their families (Evans 1971). Many of them were treated miserably, and several succumbed to prevalent diseases. Two of their kind were even executed by the Home Guard on false charges of desertion. Resentment flourished and some of the Indian men took to the swamp. There a group was formed led by Henry Berry Lowrie, which consisted of other Croatan Indians, a few negroes and some Union soldiers who escaped from the prison camp in Florence, South Carolina and were cared for by the Indians. This band prospered and they became legendary heros among the common people. They were described as being much like

Robin Hood in their exploits, being kind to the poor people and never harming those who let him and his band alone. Many of Lowrie's feats gained notoriety. After he was reported slain a Lowrie tradition was eulogized and instilled in many of the Indians (Right 1957). The legacy of Lowrie has continued, and was put to use a few years ago when some Lumbee Indians challenged and put to flight over 100 Ku Klux Klansmen who were meeting in Robeson County.

APPENDIX B

Specimens weakly reactive with Anti Rh_o

Specimen No.	Reactions with Anti- Rh_o	rh'	rh"	hr'	hr"	Most probable Wiener Genotype
38	+	0	+	+	+	R_2r
56	+	+	0	0	+	R_1R_1
59	+	+	0	+	+	R_1r
66	+	+	0	+	+	R_1r
67	+	+	0	+	+	R_1r
74	+	0	0	+	+	R_or
82	+	+	+	+	+	R_1R_2
112	+	+	0	+	+	R_1r
110	+	+	0	0	+	R_1R_1
131	+	+	0	+	+	R_1r
151	+	+	0	+	+	R_1r
155	+	0	+	+	0	R_2R_2

The reaction of all 12 specimens was enhanced by performing the D^u test. Reactions went from a weak $\pm$ to greater than 2+ in all cases.

APPENDIX C

Rh phenotypes with corresponding genotypes

Phenotypes	Corresponding genotype
rh	rr
rh'	r'r' and r'r
rh"	r"r" r"r
rh'rh"	r'r"
Rh_o	R^oR^o and R^or
Rh_1	$R'R'$, $R'r'$, $R'r$, $R'R^o$ and R^or'
Rh_2	R^2R^2, R^2r'', R^2r, R^2R^o and R^or''
Rh_1Rh_2	$R'R^2$, $R'r''$, and R^2r'

APPENDIX D

Rh Gene Frequency Formulas[a]

$$r = \sqrt{rh}$$

$$r' = \sqrt{rh' + rh} - \sqrt{rh}$$

$$r'' = \sqrt{rh'' + rh} - \sqrt{rh}$$

$$R_o = \sqrt{Rh_o + rh} - \sqrt{rh}$$

$$R_1 = \sqrt{Rh_1 + rh' + Rh_o + rh} - \sqrt{rh' + rh} - \sqrt{Rh_o + rh} + \sqrt{rh}$$

$$R_2 = \sqrt{Rh_2 + rh'' + Rh_o + rh} - \sqrt{rh'' + rh} - \sqrt{Rh_o + rh} + \sqrt{rh}$$

[a]Wiener and Wexler (1958)

APPENDIX E

Gene Frequency calculations for the MN system

Phenotype	Number	Frequency
M	15	.3061
MN	30	.6123
N	04	.0816

$$L^m = M + \frac{MN}{2}$$

$$L^n = N + \frac{MN}{2}$$

$$\text{Gene M} = .3061 + \frac{.6123}{2} = 0.6122$$

$$\text{Gene N} = .0816 + \frac{.6123}{2} = 0.3878$$

Expected MM = $(.6122)^2 = 0.3748$

Expected MN = $.6122 \times .3878 \times 2 = 0.4748$

Expected NN = $(.3878)^2 = 0.1504$

		Expected	Observed
M	0.3748x49 =	18.4	15
MN	0.4748x49 =	23.3	30
N	0.1504x49 =	7.4	04

$$X^2_{(1)} \quad \frac{(15-18.4)^2}{18.4} + \frac{(30-23.3)^2}{23.3} + \frac{(7.4-4)^2}{7.4} = 4.11$$

$.025 < P < .05$

APPENDIX F

Sample Questionaire

DUKE UNIVERSITY MEDICAL CENTER
DIVISION OF IMMUNOLOGY
DURHAM, N. C. 27710

Family name:________________________ date__________________________

Mother   # given

Full name

date of birth

deceased?
date
place

Mother's name   living?

Father's name   living?

Father

Full name   # given

date of birth

deceased?
date
place

Mother's name   living?

Father's name   living?

Other pregnancies not resulting in live birth:

Date	approx. order to other births	cause (Miscarriage, abortion, stillbirth)

Are father and mother related? In what way?(1st or 2nd cousins, uncle, niece)

Is there any mental retardation in the family? Diagnosis?

Any congenital malformations? Diagnosis?

Any known heredity conditions?

Additional comments:

Offspring of Mother:

Full name	birthdate	deceased	cause	spouse's full name	# given